

UNIVERSITY OF PENNSYLVANIA

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HIGHER PLANTS

LEWIS EDWARD ANDERSON

A DISSERTATION

IN BOTANY

PRESENTED TO THE FACULTY OF THE GRADUATE SCHOOL IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

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Reprint from
The American Journal of Botany, Vol. 23, No. 7

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Lewis E. Anderson

THE BEHAVIOR of cytoplasm and cytoplasmic inclusions in the life cycles of plants has received very little attention, and up to the present the genetical data which indicate that the cytoplasm may serve as the bearer of certain heritable characters have been supported by only meager cytological evidence. Chlorophyll deficiencies which do not follow regular Mendelian ratios, and which may accordingly be inferred to be inherited in a manner other than through the chromosomes, have been found in approximately forty species of plants. It is therefore desirable that additional knowledge be secured of the behavior of any possible bearer of the factors concerned.

It is perhaps significant that such a large proportion of these non-Mendelian characters should be concerned with plastids, and the question arises as to the possibility of a direct transmission of these cell organs or of their primordia from one generation to the next. In certain algae fully developed chloroplasts pass directly into the egg and thus into the daughter plant; but in seed plants the chloroplasts as such do not persist through all phases of the life cycle, although they may be represented in certain stages by their primordia. The purpose of this investigation is to trace the behavior of these bodies through the entire life cycle, especially during the development of reproductive structures and the processes leading to fertilization. It is hoped that such a study may throw some light on the material basis of maternal inheritance.

Cytologists, in general, agree that plastids arise from some visible primordium in the cytoplasm, the transition of which has been figured many times. There is much confusion, however, in the terms used to designate these primordia, and most of the disagreement as to what constitute plastid primordia is probably due to this confusion of terms. All cytoplasmic inclusions (plastid primordia and other bodies) preserved by certain "basic" fixatives exhibit similar staining qualities; and thus it is not possible at present to distinguish between these primordia and other similar appearing bodies—if, indeed, any such distinction actually exists. Until we are able to identify definitely the bodies which give rise to plastids and those which do not, it seems best to use some inclusive term to embrace them all. For convenience, therefore, the term "mitochondria" will be used for

all those small cytoplasmic inclusions preserved by bichromates on the basic side of pH 4.2–5.2 and destroyed by more acid fixatives and by mixtures of bichromates and acetates. This term thus includes the primordia of plastids, whereas the term "plastid" itself will include only those bodies containing starch or chlorophyll.

The literature bearing on the genetics of chlorophyll deficiencies has been extensively reviewed by de Haan (1933) and East (1934). It is necessary here to review, briefly, only phases of the cytology of the work which concerns this investigation. The cytology of variegated plants has been investigated by a few workers. Miles (1915) examined albino seedlings of *Zea Mays* and found no evidence of plastids in pure white plants. Randolph (1922) made rather extensive studies on several types of chlorophyll deficiencies in the same species and reported that all types contain minute proplastids. In contrast to Miles' findings, white seedlings were found to contain plastids which were retarded in their development. Randolph found only one type of plastid primordia in meristematic tissue and no differences were found between green and colorless plastids. Zirkle (1929) found that mitochondria in the root tips of *Zea Mays* developed into plastids, all stages of development being figured. In addition, he found two types of mitochondria, one of which develops into plastids, the other, a smaller type, apparently undergoing no change. In the stem growing point the primordia are usually minute plastids, although occasionally they are mitochondria. In variegated plants of *Zea* exhibiting maternal inheritance, Zirkle distinguished two kinds of plastids in lighter and darker stripes of the leaf with no intermediate forms on the borderline of the two regions. No evidence was presented, however, that these two types of plastids are derived from two distinct classes of primordia. Tsinen (1923), working with *Abutilon*, maintained that variegation is due to changes in the plastid primordia or the plastids themselves. In *Hosta*, Yasui (1930) could not distinguish between primordia of green, yellow, and white plastids, nor did she find intermediate forms in mature cells.

The presence of plastids in the egg has been long known (Schimper, 1885), and more recently Guilliermond (1924) described proplastids or mitochondria in the egg of *Lilium*. Mitochondria have been figured in the pollen grains of several plants, among which are *Cucurbita Pepo* (Guilliermond, 1912) and *Helleborus* (Wagner, 1927), as well as in the spores of *Equisetum* (Lewitsky, 1925). In the pollen tube of *Lupinus luteus*, Ruhland and Wetzel (1924) observed structures resembling mitochondria which originated from plastids in the pollen grain. The transfer of any of these cytoplasmic bodies from the pollen

¹ Received for publication May 16, 1936.

Contribution from the Botanical Laboratory and the Morris Arboretum of the University of Pennsylvania.

This investigation was suggested by and carried on under the direction of Prof. Conway Zirkle, to whom the writer wishes to express his sincere thanks and appreciation. Prof. Edgar T. Wherry and Prof. John M. Fogg, Jr., have rendered helpful assistance in the preparation of the manuscript. The work was done while the writer held a Morris Arboretum fellowship.



tube to the egg, however, has not been demonstrated cytologically.

MATERIALS AND METHODS.—The plants investigated were *Antirrhinum majus* L., *Philadelphus coronarius* L., and *Hyacinthus orientalis* L. Flowers of *Hyacinthus* were obtained from bulbs planted in the greenhouse and forced during the months of December and January, at which time they flowered freely. Root tips, stem growing points, anthers, and ovaries in various stages of development were fixed at intervals. Specimens of *Antirrhinum* were likewise obtained from plants grown in the greenhouse from seed, those of *Philadelphus* from plants growing at the Morris Arboretum. In order to obtain the various fertilization stages, the plants were hand-pollinated and the rate of pollen-tube growth was determined by smearing the styles on a slide at various intervals after pollination. The specimens were then stained in crystal violet for 30 seconds and washed in water, the excess stain being removed with filter paper. The pollen tubes stain a brilliant violet while the stylar tissue stains only faintly. Pollen tubes could thus be traced to the micropyles, at which time the ovaries were fixed at two-minute intervals until fertilization had occurred. Pollen tubes were fixed directly in the style as well as on glucose agar where they had been germinated. In the latter case small blocks of agar upon which they were growing were cut out, plunged into the fixative, imbedded in paraffin, and sectioned in the usual manner.

In order to preserve mitochondria, it was necessary to use bichromate fixatives on the basic side of the pH range 4.2–5.2, a number of which were employed. By far the most satisfactory fluid, however, was Zirkle's (1934) modification of Erliki's potassium bichromate-copper sulphate mixture in the following proportions: $K_2Cr_2O_7$, 1.25 g.; $(NH_4)_2Cr_2O_7$, 1.25 g.; $CuSO_4$, 1.00 g.; H_2O , 200.00 g. Enough pyridine was added to this mixture to make $\frac{1}{4}$ of 1 per cent. After the resulting precipitate had settled (which required about 20 minutes), specimens were placed in the fluid for 48 hours. They were then washed in water for an hour, dehydrated in butyl alcohol (Zirkle, 1930) and embedded in paraffin. Sections were generally cut 8 microns in thickness. The only stain employed was Heidenhain's iron-alum haematoxylin.

All figures were drawn at the level of the table with the aid of an Abbé camera lucida under a Spencer objective, 1.8 mm., N. A. 1.30, with either an 8× or a 20× ocular. They have been reduced approximately $2\frac{1}{2}$ times and show a magnification of about 975 diameters unless otherwise designated.

All the specimens investigated were preserved in bichromate fixatives on the basic side of the pH range 4.2–5.2. The cells accordingly show the typical "basic image," including both mitochondria and immature plastids which would have been destroyed by more acid fixatives. Unfortunately, the fixatives employed dissolve chromatin; and as a result the resting nucleus is preserved as a solid mass of nuclear lymph surrounding the heavily stained nucleolus

(fig. 1), while in dividing cells the coagulated nuclear lymph forms a spherical, dark-staining mass (fig. 7). A number of artifacts are thus found in dividing cells. No attempt was made to follow the behavior of mitochondria during nuclear division, as this would have had little direct bearing upon the subject under investigation.

MITOCHONDRIA IN APICAL MERISTEMS.—As mitochondria arise through the division of preëxisting mitochondria (Guilliermond, 1912; Friedrichs, 1922; et al.) and as they are transmitted through the dividing cells of the meristem, it is possible to trace the course of their development from the stem growing point of the young sporophyte to the micro- and megaspores and finally to the male and female gametophyte. Thus the pollen tube and the embryo sac derive their mitochondria from the same primordia, as both of these organs are developed from the same apical meristem. The mitochondria in the male and female gametes, therefore, can be traced to a common origin.

In the meristematic cells of the root the mitochondria assume various forms, comprising both straight and curved rods, and granules or chains of granules (fig. 1). In *Hyacinthus* they are clustered about the nucleus and around the periphery of the cell, although some are also scattered throughout the cytoplasm. Mitochondria which fix as hollow spheres are not uncommon and their transition into plastids can be followed by tracing the intermediate forms from the apex of the root to the region of differentiation, where they become mature plastids (fig. 2). This transition is similar to that described by Zirkle (1929) for root tips of *Zea Mays*.

Longitudinal sections of stem growing points of *Hyacinthus* reveal both rod-shaped and granular mitochondria as well as small plastids, the latter increasing in number toward the region of elongation. All stages in the transition of mitochondria into plastids may be observed in the vicinity of the growing tip. Even cells with mature plastids, however, always contain some undifferentiated mitochondria. Both mitochondria and plastids are exceedingly abundant in the procambial strand and vascular bundles. Embryonic leaves also show the characteristic stages in this transition, and older leaves contain in addition mature plastids. The cells of the stem growing point of *Philadelphus* contain many minute plastids which are fixed as small hollow spheres, together with a few granular mitochondria and an occasional curved slender rod (fig. 3). There is no sharp dividing line between the plastids and the granular mitochondria, as there are many intermediate forms, particularly in cells shortly removed from the growing point (fig. 4).

Floral branches of the different plants studied show inclusions characteristic of their respective shoot meristems. Mitochondria and plastids in the meristematic protuberances originating at the tip of the young flower bud are identical with those in the more mature floral parts which arise from such protuberances. The primordia of each flower part, being

all derived from the growing point of the same floral branch, then receive the same types of cytoplasmic inclusions. Thus it is evident that there is an equal distribution of mitochondria to microspores and megaspores; and even if there should be more than one kind of mitochondria, as Meyer (1920), Bowen (1927, 1929), Ruhland and Wetzel (1924), and others have reported, there is no evidence of any segregation of different types in the two kinds of spores.

MITOCHONDRIA IN MICROSPORES AND MALE GAMETOPHYTE.—The microspore mother cells of *Philadelphus* and *Antirrhinum* are extremely small, the nucleus occupying the greater part of each cell (fig. 5). This is particularly evident after meiosis is initiated, for at that point the nucleus increases in size perceptibly (fig. 7). The cytoplasm forms a relatively thin layer about the nucleus and the periphery of the cell, with vacuoles forming the greater part of the cytoplasmic volume. Plastid primordia are numerous and are preserved by the fixatives as large spherical dark-staining bodies, although there are in addition some smaller spheres and rods (fig. 5). Mitochondria in the process of transformation into plastids surround some of the nuclei, while minute plastids already transformed are not uncommon. The plastids fix as hollow spheres with a minute starch grain in the center. Transitional stages become more abundant as meiosis proceeds.

In *Hyacinthus* the mother cells are much larger than in the two above-mentioned genera, although the nuclei are also correspondingly larger and occupy most of the cell (fig. 6). The cytoplasm is strikingly vacuolate and is densely filled with mitochondria which stain intensely. They fix generally as solid spheres and short thick rods, although there are occasional slender threads. In mother cells there are apparently no transitional stages between mitochondria and plastids. The cytoplasm becomes increasingly vacuolate as division proceeds (fig. 7). Mitochondria are transmitted through the reductional divisions directly to the microspores, and each microspore apparently receives an approximately equal share of the cytoplasmic inclusions. There is a very striking increase in the number of plastid primordia in the microspore over those appearing in the sporocyte (cf. fig. 7, 18). Whether this increase is due to an actual multiplication of these bodies or whether they represent mitochondria formerly preserved below the limit of visibility is not certain. The latter theory, however, seems less probable, since at no stage were mitochondria observed to grade off in size to the border line of visibility.

Mitochondria are exceedingly abundant in the mature microspore or pollen grain just before it is shed and are preserved as short, thick, solid rods and spheres which have increased somewhat in size (fig. 18). The cytoplasm is very vacuolate, leading to the clumping of the mitochondria into definite aggregations or bundles, a normal condition in relatively scanty cytoplasm (fig. 21). In the mature pollen grains at the time of shedding both the larger

solid rod-shaped mitochondria and the larger spheres begin to appear hollow (fig. 21). At this time the transition into plastids occurs, and later, at the time of pollination, young plastids are numerous. The latter can be demonstrated in thin sections in which most of the mitochondria have been completely destained, for in such sections the plastids are very conspicuous (fig. 25). They are hollow spheres or rods each with a minute starch grain in the center.

Mitochondria in the microspores of *Philadelphus* and *Antirrhinum* are not nearly so abundant as in those of *Hyacinthus*, and they fix as extremely small spheres and thin straight and curved rods. Most of the inclusions are plastids. Possibly this difference in the number of the cytoplasmic inclusions in *Philadelphus* and *Antirrhinum* on the one hand and *Hyacinthus* on the other is due to variations in their fixing properties. It is also possible that substances leached out of the cells of the specimens fixed may have dissolved some mitochondria.

Pollen-tube growth is readily followed in *Hyacinthus*, owing to the shortness of the style and to the relatively large amounts of cytoplasm in the pollen tubes. Living pollen tubes were grown directly on glucose-agar, and both active cytoplasmic streaming and the passage of the nuclei down the tube could be observed. The streaming cytoplasm is filled with granules and with distinct rod-shaped bodies. The cytoplasm of living pollen grains exhibits these same inclusions, and as the germination tube makes its appearance they can be seen flowing directly from the pollen grain into the elongating tube (fig. 19). The nature of these inclusions visible in the living material is not altogether certain, but they strongly suggest mitochondria and plastids. Both mature pollen grains and pollen tubes react positively to the iodine test for starch, which is evidence of the existence of plastids.

In the pollen tube the mitochondria are fixed either as small spherical bodies or as rods, which may be either short and thick or thread-like (fig. 15, 19). Both the transitional forms of mitochondria and the plastids observed in pollen grains flow into the pollen tube with the streaming cytoplasm (fig. 19, 20). The cytoplasmic inclusions are more concentrated at the tip of the tube where they surround the tube nucleus. Each male nucleus is likewise accompanied by a dense mass of mitochondria. In *Hyacinthus* the male nuclei are not enclosed in individual cells but lie free in the pollen tube. As they migrate down the tube, they are surrounded by a characteristic mass of inclusions (fig. 15) which accompany them until they are discharged into the embryo sac. In the pollen tubes of both *Antirrhinum* and *Philadelphus* mitochondria are abundant, but they are not easily seen because of the small size of the tubes and the difficulty of fixation. Plastids were not observed in either of these genera. The mitochondria usually occur as tiny spheres and very slender rods, either straight or curved, forms which were characteristic of the microspore mother cells and growing points. The male

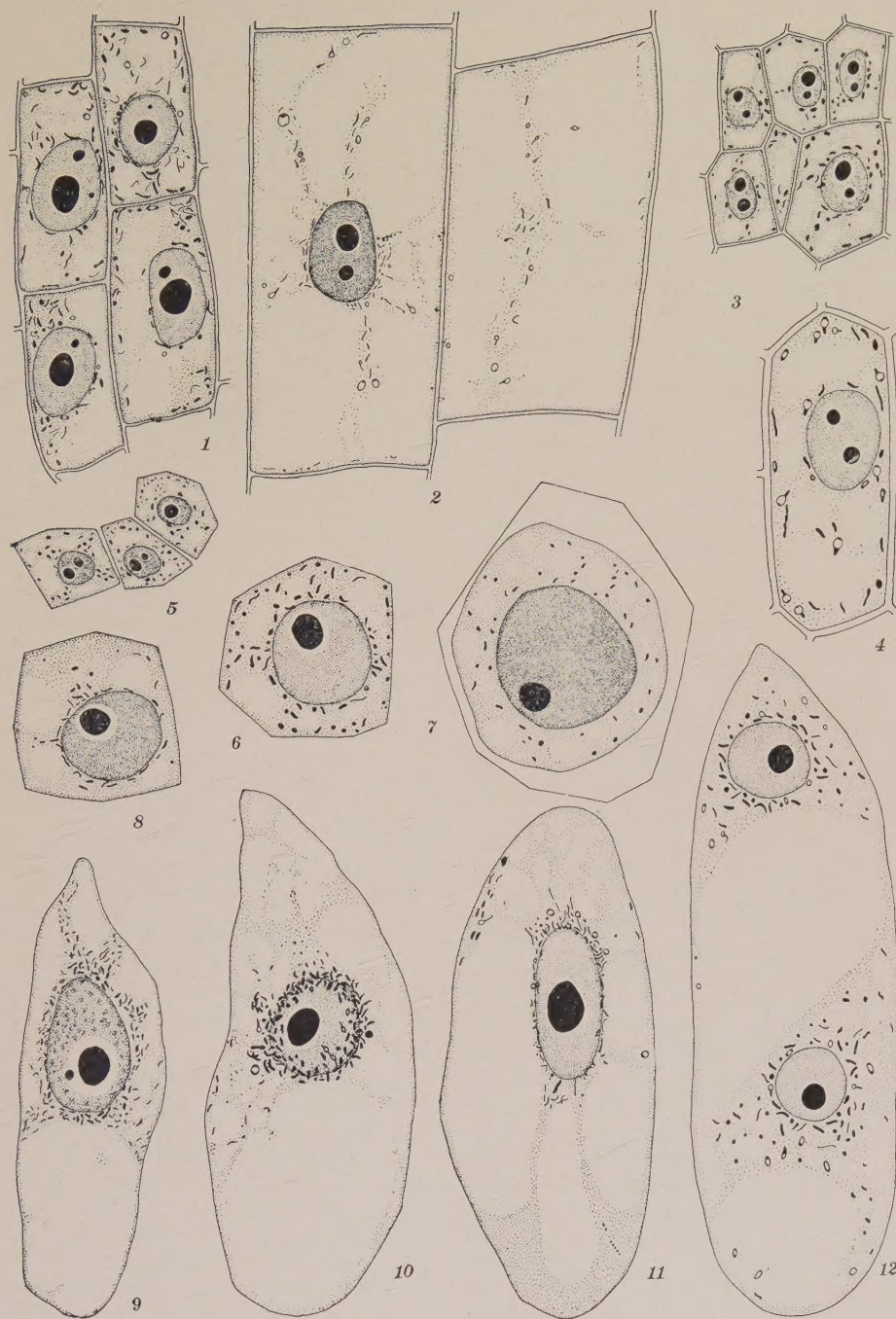


Fig. 1-12.—Fig. 1. Cells from root tip of *Hyacinthus*.—Fig. 2. Cells from the region of elongation in *Hyacinthus*, showing mitochondria, plastids, and transitional stages between the two.—Fig. 3. Cells from the stem growing point of *Philadelphus*.—Fig. 4. Cell from elongating region of stem tip of *Philadelphus*.—Fig. 5. Pollen mother cells of *Philadelphus*.—Fig. 6. Pollen mother cell of *Hyacinthus*.—Fig. 7. Microsporocyte of *Hyacinthus* during meiosis.—Fig. 8. Megaspore mother cell of *Hyacinthus*.—Fig. 9. Enlarging megaspore of *Hyacinthus*.—Fig. 10. Megaspore of *Hyacinthus*, with mitochondria grouped about the nucleus.—Fig. 11. Mature megaspore, showing the large vacuoles and characteristic cytoplasmic inclusions concentrated around the nucleus.—Fig. 12. Two-celled stage of embryo sac of *Hyacinthus*.

nuclei are spherical and extremely small, in sharp contrast to those of *Hyacinthus*, but they are similarly surrounded by mitochondria which are present as the pollen tube enters the embryo sac.

MITOCHONDRIA IN MEGASPORES AND FEMALE GAMETOPHYTE.—The cells of the meristematic protuberances which give rise to the ovules are in general similar to the premeiotic cells of the anthers, which have already been described. In *Hyacinthus* the nuclei are surrounded by both spherical and rod-shaped mitochondria and the cytoplasm contains several prominent vacuoles. There are no visible plastids or transitional forms, and all the cells of the primordia of ovules are alike until the megaspore mother cells are differentiated. At this time the nucellus has become prominent and the inner and outer integuments have formed. These structures contain an abundance of mitochondria, which collect around the nucleus and in the cytoplasmic strands between the vacuoles and just within the cell wall. The megaspore mother cell develops from one of these cells (fig. 8). It enlarges rapidly and becomes more vacuolate as meiosis is initiated. The cytoplasmic inclusions of the mother cells of *Antirrhinum* are similar to those of *Hyacinthus* except for their smaller size and predominantly granular form.

Meiosis occurs rapidly in both genera. The nuclear details are difficult to follow, however, when the material is preserved in basic fixatives, although the equal distribution of mitochondria to the four megaspores can easily be seen. In both *Hyacinthus* and *Antirrhinum* the megaspore nearest the micropylar end of the ovule develops into the embryo sac, the others disintegrating in the normal fashion. All four megaspores possess mitochondria which maintain their characteristic grouping around the nuclei. The functioning megaspore enlarges and gradually encroaches upon the other megaspores, which finally disintegrate. These disintegrating cells retain their mitochondria for a time, but eventually their staining properties become such as to obscure any inclusions that might be contained. Continued enlargement of the inner megaspore results in the others being pushed together until finally they are either obliterated or reduced to a cap at the end of the functioning megaspore. As the latter enlarges, its cytoplasm becomes increasingly vacuolate until it is limited to a thin layer about the periphery of the cell with a few strands traversing the vacuoles to connect with the centrally located nucleus (fig. 9). Mitochondria are limited almost entirely to the cytoplasm surrounding the nucleus, where they form a dense mass (fig. 10). Solid, spherical mitochondria predominate, but there are also numerous short or elongate rods and threads. Undeveloped plastids are present usually as hollow spheres and rod-shaped mitochondria which have become vacuolate at one or both ends (fig. 11).

The development of the megaspore in *Antirrhinum* differs in certain minor details from that of *Hyacinthus*. The nucleus of the enlarged megaspore is likewise surrounded by a dense mass of mitochondria

which differ from those in *Hyacinthus* only in size. In both genera the grouping of mitochondria around the nucleus is pronounced, and only scattered inclusions occur throughout the rest of the cytoplasm (fig. 10). Occasional transitional forms are present and not infrequently there are small plastids.

The megaspore enlarges perceptibly before the first division of the nucleus, after which the daughter nuclei migrate, one to each end of the embryo sac, and the cytoplasm becomes progressively more vacuolate (fig. 12). Each daughter nucleus is also surrounded by the characteristic grouping of cytoplasmic inclusions similar to the parent nucleus and each receives approximately an equal number. The four nuclei produced by the next division are all surrounded by the characteristic clumps of mitochondria (fig. 16). A third division produces eight nuclei, four at each end of the embryo sac. Each of the nuclei is surrounded by a mass of cytoplasmic inclusions which consist of mitochondria, transitional forms, and plastids. During the migration of the polar nuclei to the center of the embryo sac each nucleus retains its individual sphere of surrounding inclusions. In *Antirrhinum*, the polar nuclei fuse as soon as they come in contact with each other and a very prominent grouping of mitochondria is formed in the center of the embryo sac (fig. 22). Mitochondria are preserved generally as solid spheres which are comparatively small in *Antirrhinum* but very abundant. Rods and threads are also present but they are not so numerous as the granules. Transitional forms as well as plastids occur not infrequently. In *Hyacinthus* the two polar nuclei usually do not fuse until after the pollen tube has grown approximately half way down the style. They are both surrounded by plastid primordia, however, which are larger and more conspicuous than those in *Antirrhinum*.

The mature embryo sac in both *Hyacinthus* and *Antirrhinum* contains eight nuclei before the two polar nuclei fuse. The antipodal nuclei of *Antirrhinum* are small and are occasionally included in three somewhat inconspicuous cells, each definitely delimited by a membrane (fig. 23). Usually, however, the nuclei lie free in the embryo sac. All three nuclei are surrounded by mitochondria. In *Hyacinthus*, on the other hand, the antipodals are exceptionally large (fig. 14), with their cytoplasm very vacuolate. They are difficult to fix satisfactorily and are often so shrunken and distorted as to be scarcely recognizable. When properly fixed, however, the nucleus of each cell is surrounded by numerous mitochondria and some plastids. The polar nuclei, likewise surrounded by these inclusions, usually lie adjacent to the antipodals and are often in contact with them. The greater part of the volume of the embryo sac in both *Antirrhinum* and *Hyacinthus* is occupied by large vacuoles (fig. 22), the cytoplasm being limited to a thin layer around the periphery and to the egg apparatus, antipodals, and endosperm nuclei. The mitochondria and plastids, of course, are limited to



Fig. 13-21.—Fig. 13. Single synergid from embryo sac of *Hyacinthus*.—Fig. 14. Outline drawing of mature embryo sac of *Hyacinthus* showing (a) synergids, (b) egg, (c) fused polar nuclei, and (d) antipodals $\times 200$.—Fig. 15. Portion of a pollen tube of *Hyacinthus*, showing the two male nuclei surrounded by mitochondria. The tube nucleus is shown at the tip.—Fig. 16. Four-celled stage of embryo sac of *Hyacinthus*.—Fig. 17. Egg of *Hyacinthus* showing plastids and mitochondria surrounding the female nucleus.—Fig. 18. Young microspore of *Hyacinthus*.—Fig. 19. Germinating pollen grain of *Hyacinthus* showing mitochondria and plastids flowing into the pollen tube.—Fig. 20. Portion of a pollen tube destined to show plastids and transitional stages between mitochondria and plastids.—Fig. 21. Mature pollen grain of *Hyacinthus*. Mitochondria appear clumped owing to vacuoles.

the cytoplasm and are most abundant in the regions adjacent to the nuclei, although scattered inclusions are present throughout the cytoplasm of the entire embryo sac. The cells of the nucellus contain few vacuoles and stain heavily. These cells contain more mitochondria than any other cells of the ovule; in fact, in *Antirrhinum*, the nucellus is practically filled with small granular mitochondria (fig. 22). The cells of the integuments also contain mitochondria, but they are less abundant than in the nucellus.

In fixed material of both *Antirrhinum* and *Hyacinthus* the cells of the egg apparatus as well as the synergids are bounded by distinct membranes (fig. 13, 17, and 22), and consequently their cytoplasm is definitely separated from the rest of the female gametophyte. The two synergids lie one on each side of the egg, which extends somewhat more deeply into the embryo sac. The nucleus of each synergid is located at the center of the cell and is completely surrounded by mitochondria of the same nature as those in the microspore. The cytoplasm is denser in the region toward the micropyle and becomes more vacuolate at the other end, usually with a single large vacuole filling most of the broader extremity of the egg (fig. 13, 22). In *Hyacinthus* the synergids are somewhat larger than the egg, with mitochondria very abundant in the nuclear region but less numerous toward the micropylar end (fig. 13). The synergids are smaller in *Antirrhinum* and in closer contact with the egg cell. The nuclei are very small and inconspicuous, and both plastid primordia, which are preserved as rods and solid or hollow spheres, and small plastids are present (fig. 22).

The nucleus of the egg of *Hyacinthus* is large and conspicuous and lies in the end away from the micropyle (fig. 14). The surrounding cytoplasm is rather dense, becoming more vacuolate toward the micropylar end (fig. 17). Mitochondria are likewise most numerous around the nucleus, forming the characteristic dense mass already described in the pollen tube and other structures. They are preserved as solid spheres, thick rods, or threads, and any of these forms may serve as the primordia of plastids. These are not uncommon in the eggs of both *Antirrhinum* and *Hyacinthus* and develop in the same general manner in both species (fig. 22). The mitochondria in the egg of *Antirrhinum* are almost as numerous as they are in *Hyacinthus*, but they are much smaller and appear mostly as spheres and short rods. A similar transition into plastids occurs in this species, although the hollow spheres are much more difficult to distinguish and the resulting plastids are correspondingly smaller (fig. 27).

MITOCHONDRIA IN FERTILIZATION.—Actual fertilization was observed only in *Antirrhinum*, which, because of the large number of ovules in a single ovary, is relatively easy to find. As the pollen tube enters the embryo sac, the tube nucleus is surrounded by mitochondria. The tube passes between the synergids and lies along the side of the egg where, in some cases, it may remain for some time before it ruptures.

The male nuclei pass down the tube, each nucleus being surrounded by mitochondria. The pollen tube after entering the embryo sac becomes much swollen and moves toward the region of the egg between the female nucleus and the micropyle (fig. 23). A protuberance appears on the unruptured pollen tube and presses against the egg. The mode of rupture of the tube was not determined, but it apparently occurs rapidly and with considerable force as described by several previous investigators. No perforation such as that reported by Schaffner (1897) and others was observed. The male nucleus which is to fuse with the polar nuclei is apparently discharged first, and migrates through the cytoplasm of the embryo sac (fig. 23), finally fusing with the polar nuclei to form the endosperm nucleus (fig. 24). In the course of this migration through the cytoplasm it is surrounded by mitochondria (fig. 23), although it was not possible to determine with certainty whether these surrounding inclusions were discharged along with it from the pollen tube or whether they were originally present in the cytoplasm of the embryo sac. Probably, however, some cytoplasm escapes from the pollen tube at the time of rupture, for this breaking is apparently due to the high osmotic pressure in the tube, and in view of the large number of mitochondria in the cytoplasm it is hardly conceivable that at least a portion of them can fail to pass into the embryo sac along with this male nucleus.

The male nucleus which is to unite with the egg nucleus enters the egg immediately after it is discharged from the pollen tube. In a preparation such as that shown in figure 23, there seems to be an actual connection between the pollen tube and the egg. If such is the case, this opening would afford ample opportunity for the passage of plastid primordia from the tube to the egg. Furthermore, since mitochondria are so numerous around the male nucleus during its migration down the pollen tube, it is difficult to see how, under such conditions, the nucleus could enter the egg without at least part of these inclusions accompanying it. After the male nucleus has entered the egg, it is surrounded by mitochondria which are similar to those accompanying it in the pollen tube (fig. 23). To be sure, the actual passage of mitochondria from the pollen tube into the egg was not observed. Accordingly, it cannot be stated definitely that these mitochondria, which surround the male nucleus in the egg cell, were discharged along with it when the pollen tube ruptured, for no mitochondria or other inclusions present in the egg after fertilization could be definitely identified as having been passed into it from the tube along with the male nucleus. However, since the male nucleus is surrounded by mitochondria just before it is discharged and the cytoplasm of the entire pollen tube is filled with inclusions, it would seem impossible for the pollen tube to discharge its nuclei without some of these inclusions escaping into the embryo sac. Since the polar nuclei are surrounded by no special cell membrane or wall (fig. 22), the

resulting endosperm will almost certainly contain some mitochondria which were released from the pollen tube. Since the opening through which the male nucleus passes from the tube into the egg is sufficiently large to permit the entrance of inclusions along with it (fig. 23), there seems no reason to doubt that they do enter. As the male nucleus migrates through the cytoplasm of the egg to the egg nucleus, it is surrounded by both mitochondria and plastids. After fusion, the fertilized egg nucleus is likewise surrounded by many small granular mitochondria and some scattered hollow spheres and small plastids (fig. 24). The ruptured pollen tube still contains a few mitochondria, although they are not nearly so numerous as they were before the male nuclei were discharged.

MITOCHONDRIA IN THE EMBRYO.—After fertilization, both the diploid nucleus of the egg and the triploid endosperm nucleus are surrounded by mitochondria, plastids, and transitional forms between the two (fig. 24). The endosperm nucleus divides immediately and a wall is laid down dividing the two daughter cells, both of which contain mitochondria and plastids. Further development of the endosperm is rapid, walls being formed after each division and each nucleus in turn being surrounded by mitochondria. The endosperm cells are large and highly vacuolate, the cytoplasm being limited to the nuclear region and to a thin layer just within the cell wall, in which there are likewise scattered inclusions (fig. 26). The endosperm contains mature plastids, as can be seen in figure 26. The fertilized egg does not divide until the development of the endosperm is well advanced, but remains a single, large, vacuolate cell, with mitochondria scattered through the cytoplasm and characteristically clustered about the nucleus (fig. 26). After the endosperm forms a many-celled structure, the nucleus of the fertilized egg divides (fig. 27), forming a two-nucleate structure at the end of the endosperm, after which a wall is laid down. Subsequent divisions result in the formation of an elongated row of cells constituting the proembryo, all of which possess mitochondria derived from the fertilized egg. Further differentiation results in the development of the suspensor and of the embryo proper, which contain cytoplasmic inclusions in no way different from those of the fertilized egg. The terminal cells of the embryo continue their division, forming the cotyledons and growing points, into which are transmitted the identical inclusions which were present in the fertilized egg.

DISCUSSION.—Geneticists, in general, have agreed that the inheritance of certain chlorophyll abnormalities which do not conform with regular Mendelian ratios are conditioned by the cytoplasm or its inclusions. Hence, certain chlorophyll deficiencies which are transmitted only through the female plant have been explained by the assumption that the egg does not receive any male cytoplasm during fertilization. This explanation was first suggested by Correns in 1909, when he assumed that the transmission of varie-

gation in *Mirabilis*, the classical example of maternal inheritance, was through the cytoplasm, since the variegation was not carried by the male nucleus. He assumed further that this character is transmitted only through the egg cell, for he believed that no cytoplasm enters the egg with the sperm nucleus. The development of the "defective" plastids which cause variegation was believed by Correns (1928) to be conditioned by diseased cytoplasm, a theory which has the recent support of East (1934) in a critical review of the subject. Other investigators (Gregory, 1915; Winge, 1919; Chittenden, 1925; et al.) postulated that the plastids themselves or their primordia are the bearers of the deficiency. Baur (1909), on the other hand, explained the non-Mendelian variegation in *Pelargonium zonale*, which is inherited through both parents, by assuming that plastids were brought into the egg cell along with the male nucleus, since this character is transmitted by both parents. There are other genetical data indicating that male cytoplasm enters the egg in fertilization (see East, 1934).

The investigations of the writer do not support the view that no male cytoplasm passes into the egg during fertilization. The persistence of plastids or their primordia during all phases of the life cycle can easily be demonstrated. They exist in the apical meristems and in all the organs derived from the stem growing points. They assume identical forms in both the male and female gametophytes, where they are exceedingly numerous. They are especially concentrated in the cytoplasm of the pollen tube, where they surround both male nuclei, and, as the tube is ruptured, they are expelled into the cytoplasm of the embryo sac. The resulting endosperm, therefore, contains plastids contributed by both parents. These relations have not been recognized heretofore, because nearly all previous investigators of this problem have been limited to fixed cytological preparations where the cytoplasm has been preserved in such a manner that all its inclusions were dissolved.

The egg itself, on the other hand, is delimited by a distinct membrane. Whether or not the fertilized egg contains plastids contributed by the male plant will depend upon the ability of this membrane to divest the male nucleus of its surrounding cytoplasmic inclusions. The exact mode of entrance of the male nucleus could not be determined. Heimans (1928) reported that the male gamete enters the egg cell through a small opening in the membrane, which may well be, in view of the observations made by the writer. After the male nucleus has entered the egg, the opening through which it has passed remains for a time in the membrane (fig. 23). This pore is large enough to permit mitochondria to enter along with the male nucleus, and as shown in figure 23, they are present both inside the egg and in the adjacent pollen tube. In view of these observations it is difficult to conceive of the male nucleus entering the egg without a certain number of mitochondria as well as some cytoplasm accompanying it. As the mitochondria of the pollen tube do not differ in present staining re-

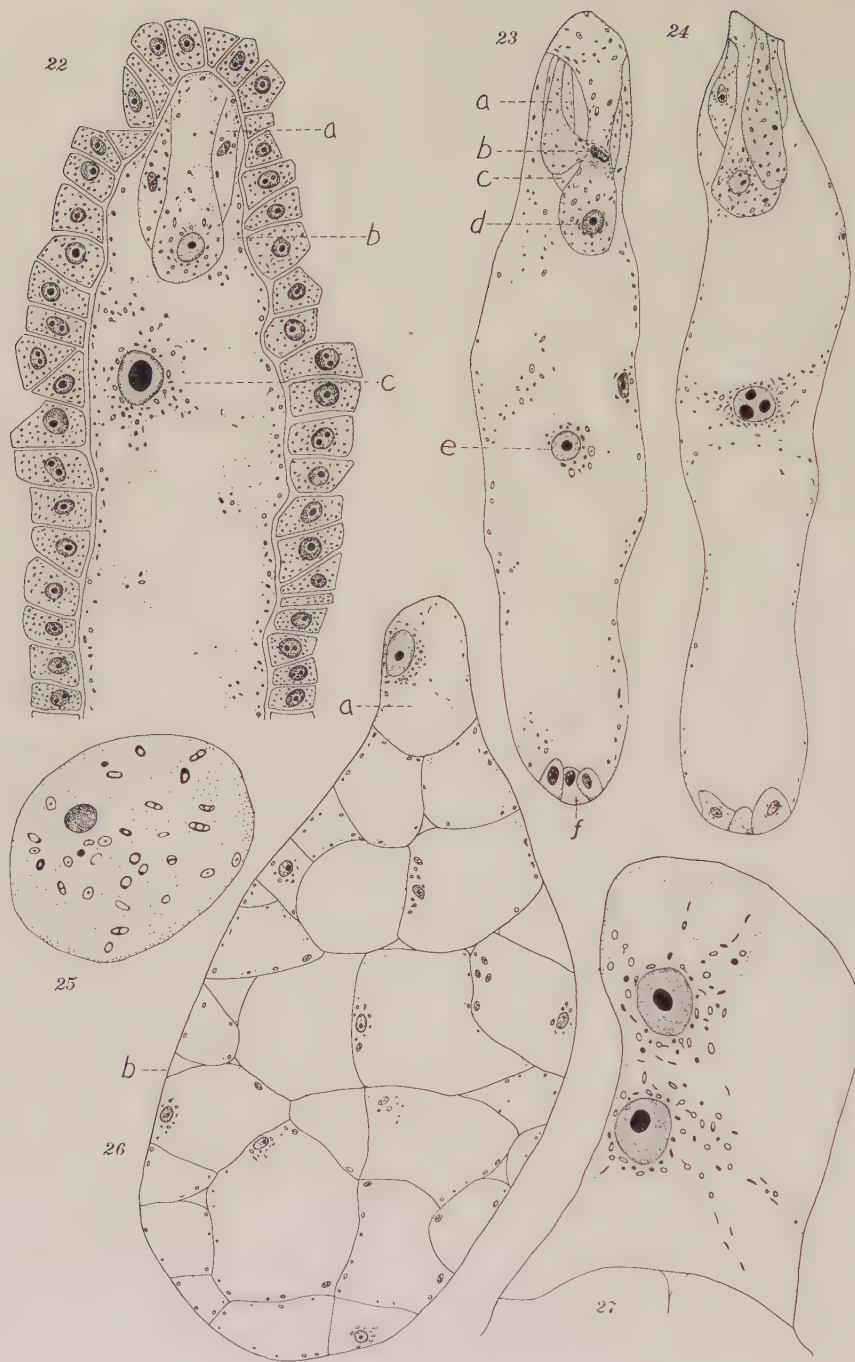


Fig. 22-27.—Fig. 22. Micropylar end of mature embryo sac of *Antirrhinum*, with surrounding nucellus; (a) synergid, (b) egg, (c) fused polar nuclei.—Fig. 23. Embryo sac of *Antirrhinum* just after the pollen tube has ruptured. One male nucleus (b) has entered the egg leaving an opening in the membrane. The other male nucleus is migrating through the cytoplasm of the embryo sac to the fused polar nuclei (e). Other structures shown are (a) pollen tube, (c) synergid, (d) egg nucleus, (f) antipodals.—Fig. 24. Embryo sac of *Antirrhinum* immediately after fertilization, showing plastids and transitional forms.—Fig. 25. Mature pollen grain of *Hyacinthus* with mitochondria destined to show plastids and transitional forms.—Fig. 26. Embryo sac after fertilization, showing endosperm (b), and zygote (a) which has not yet divided. $\times 412$.—Fig. 27. Young embryo showing first division of fertilized egg nucleus. The endosperm is not shown. $\times 1050$.

actions and in form from those of the egg, they cannot be distinguished from the latter once they have entered the egg.

If the cytoplasm with some of its inclusions passes into the egg along with the male nucleus, Correns' original explanation of maternal inheritance will have to be abandoned, and the cause of the failure of the pollen to transmit these plastid deficiencies will have to be sought elsewhere. In some forms (*Zea*, *Oryza*, *Hosta*, etc.) only two types of plants reach maturity, those which are variegated and those normally green. The lighter plants die. The variegated plants produce a certain amount of normal pollen—i.e., pollen without any defective plastids. When the flower is pollinated with a mixture of normal and defective pollen, selective fertilization could explain the failure of the male parent to transmit the defect.

It is evident that only a few mitochondria enter the egg with the male nucleus. They are scarce, therefore, in comparison with the large number already present in the egg cell. In the subsequent divisions of the fertilized egg which form the proembryo, these few defective primordia would have little chance of being distributed to just those cells which form the embryo proper, and even if they reached the embryo they might not be in those cells which give rise to the shoot meristem. Thus the defective plastid primordia might well participate in the act of fertilization and yet have little influence upon the progeny.

An alternate explanation can be found in the assumption that the cytoplasmic inclusions of the pollen tube do not function in the egg cytoplasm. Since any plastid primordia which may be contributed by the male cytoplasm during fertilization are indistinguishable from those already present in the egg, the plastid primordia from the pollen tube cannot be traced as such in the subsequent development of the zygote. Consequently, there is no way of determining whether mitochondria derived from the male parent develop normally after they come in contact with the cytoplasm of the egg or whether they are incapable of developing into plastids after they enter the egg. Large numbers of mitochondria are transformed into plastids in the fertilized egg and during its immediate divisions (fig. 27), but none of these can be recognized as having been derived from the pollen tube. Any mitochondria from the male cytoplasm which enter the egg may disintegrate, and thus the primordia of defective plastids from the male plant would not be evident in the offspring. There is evidence of a parallel occurrence in certain of the green algae. In *Zygnema*, for example, the chloroplasts contributed by the "male" gamete in conjugation degenerate in the zygospore, only the chloroplasts of the female gamete remaining functional (Kurssanow, 1911). A somewhat similar development has been reported for *Spirogyra* (Tröndle, 1911). Whether the same sort of thing occurs in the higher plants cannot be determined until some method of distinguishing between the cytoplasmic inclusions of the pollen tube and of the egg has been devised.

There is, of course, the possibility that the development of normal plastids is inhibited by either diseased or defective cytoplasm, and that the factors which determine aberrant chlorophyll are not transmitted in any visible structure. Such an assumption does not markedly affect the nature of the problem, however, since the distribution of the cytoplasm and of the cytoplasmic inclusions is essentially the same.

SUMMARY

The plants investigated were *Antirrhinum majus* L., *Philadelphus coronarius* L., and *Hyacinthus orientalis* L.

It was possible to demonstrate mitochondria and plastids in structures in which they had not heretofore been reported by using fixatives on the basic side of pH 4.2-5.2. The more acid fixatives employed by most previous investigators dissolve them.

The term "mitochondria" is used to include plastid primordia, since all these groups of inclusions exhibit similar fixing and staining properties and are indistinguishable from one another with present methods of investigation.

All living plant cells studied were found to contain the primordia of plastids, the development of which can be traced in the stem growing point and in the leaf cells. Subsequent organs developed from growing points contain these primordia.

Plastid primordia are present in microspore mother cells from which they are distributed approximately equally to the microspores, where they are particularly abundant. Transition of these primordia into plastids could be followed in both pollen grains and pollen tubes. These cytoplasmic inclusions are carried from the microspores into the pollen tube by the streaming cytoplasm. They are particularly numerous about the male nuclei and persist at least until the pollen tube ruptures in the embryo sac.

Megaspore mother cells contain mitochondria identical in appearance with those of the microspore mother cells, and they are likewise distributed equally to the four megaspores. The functional megaspore possesses mitochondria which are strikingly abundant around its nucleus. The nucleus of each daughter cell is in turn surrounded by mitochondria. The egg cell contains both plastids and mitochondria, as do all the other cells of the embryo sac. The polar nuclei are surrounded by these inclusions before and after they fuse.

Fertilization was observed in *Antirrhinum*. The pollen tube does not rupture until some time after it has entered the embryo sac; instead, it remains for a time in contact with the egg. A protrusion is formed toward the egg, and at that point the tube ruptures.

The male nucleus which fuses with the polar nuclei is surrounded by mitochondria, and the resulting endosperm consequently contains cytoplasmic inclusions from the male plant. The second male nucleus, surrounded by mitochondria, enters the egg and fuses with the egg nucleus. The membrane surrounding

the egg, after it has been punctured by the male nucleus, contains an opening large enough for mitochondria to pass in from the pollen tube.

Plastids and their primordia are present in all the cells of the developing embryo where the transitional stages between mitochondria and plastids are most abundant. These inclusions are found in the cytoplasm of all cells formed before the growing points appear, as well as in the growing points themselves.

It is shown that if mitochondria from the male cytoplasm enter the egg cell, any defective cytoplasm

or cytoplasmic inclusions present in the pollen tube would be transmitted to the egg.

The explanations of maternal inheritance which assume that no cytoplasm is transferred from the male plant to the female plant in fertilization are thus inadequate. Several alternate possibilities are suggested.

DUKE UNIVERSITY,
DURHAM, NORTH CAROLINA

LITERATURE CITED

- BAUR, E. 1909. Das Wesen und die Erbliehkeitsverhältnisse der "Varietates albomarginatae hort" von *Pelargonium zonale*. Zeitschr. Ind. Abst. Vererb. 1: 330-351.
- BOWEN, R. H. 1927. A preliminary report on the structural elements of the cytoplasm in plant cells. Biol. Bull. 53: 179-196.
- . 1929. Studies on the structure of plant protoplasm. II. The plastidome and pseudochondriome. Zeitschr. Zellf. Mikro. Anat. 9: 1-65.
- CHITTENDEN, R. J. 1925. Studies in variegation. II. *Hydrangea* and *Pelargonium*: with notes on certain chimerical arrangements which involve sterility. Jour. Genetics 16: 43-61.
- CORRENS, C. 1909. Vererbungsversuche mit blass (gelb) grünen und buntblättrigen-Sippen bei *Mirabilis jalapa*, *Urtica pilulifera*, und *Lunaria annua*. Zeitschr. Ind. Abst. Vererb. 1: 291-329.
- . 1928. Über nichtmendelnde Vererbung. Zeitschr. Ind. Abst. Vererb. (Suppl.) 1: 131-138.
- EAST, E. M. 1934. The nucleus-plasma problem. Amer. Nat. 63: 289-303; 402-439.
- FRIEDRICH, G. 1922. Die Entstehung der Chromatophoren aus Chondriosomen bei *Helodea canadensis*. Jahrb. Wiss. Bot. 61: 430-458.
- GREGORY, R. P. 1915. On variegation in *Primula sinensis*. Jour. Genetics 4: 305-321.
- GUILLIERMOND, A. 1912. Recherches cytologiques sur le mode de formation de l'amidon sur les plantes végétaux. Archiv. Anat. Microscop. 14: 209-428.
- . 1924. Recherches sur l'évolution du chondriome pendant le développement du sac embryonnaire et des cellules mères des grains de pollen dans les Liliacées et sur la signification des formations ergastoplasmiques. Ann. Sci. Nat. Bot. X, 6: 1-52.
- HAAN, H. DE. 1933. Inheritance of chlorophyll deficiencies. Bibliogr. Genetica 10: 357-416.
- HEIMANS, J. 1928. Chromosomen und Befruchtung bei *Lilium Martagon*. Rec. Trav. Bot. Néerl. 25A: 138-167.
- KURSSANOW, L. 1911. Über Befruchtung, Reifung und Keimung bei *Zygnuma*. Flora 104: 65-84.
- LEWITSKY, G. A. 1925. Die Chondriosomen in der Gonogenese bei *Equisetum palustre* L. Planta 1: 301-316.
- MEYER, A. 1920. Morphologische und Physiologische Analyse der Zelle der Pflanzen und Tiere. Jena.
- MILES, F. C. 1915. A genetic and cytological study of certain types of albinism in maize. Jour. Genetics 4: 193-214.
- RANDOLPH, L. F. 1922. Cytology of chlorophyll types of maize. Bot. Gaz. 73: 337-375.
- RUHLAND, W., AND K. WETZEL. 1924. Der Nachweis von Chloroplasten in den generativen Zellen von Pollenschläuchen. Ber. Deutsch. Bot. Ges. 42: 3-14.
- SCHAEFFNER, J. H. 1897. Contribution to the life history of *Sagittaria variabilis*. Bot. Gaz. 23: 252-273.
- SCHIMPER, A. F. W. 1885. Untersuchungen über die Chlorophyllkörper und die ihnen homologen Gebilde.
- TRÖNDLE, A. 1911. Über die Reductionsteilung in den Zygoten von *Spirogyra* und über die Bedeutung der Synapsis. Zeitschr. Bot. 3: 593-619.
- TSINEN, S. J. 1923. Recherches histologiques et cytologiques sur la Panachure dans le genre *Abutilon*. Bull. Soc. Sci. Nancy IV, 2: 25-26.
- WAGNER, N. 1927. Sur la formation "de novo" des chondriosomes dans la cytoplasme des cellules-mères des grains de pollen chez les angiospermes. Biol. Generalis 3: 329-346.
- WINGE, Ö. 1919. On the non-Mendelian inheritance in variegated plants. Compt. Rend. Trav. Lab. Carlsberg 34: 1-20.
- YASUI, K. 1930. Studies on the maternal inheritance of plastid characters in *Hosta japonica* Aschers et Graeb. f. *albomarginata* Mak. and its derivatives. Cytologia 1: 192-215.
- ZIRKLE, C. 1929. Development of normal and divergent plastid types in *Zea Mays*. Bot. Gaz. 88: 186-203.
- . 1930. The use of n-butyl alcohol in dehydrating woody tissue for paraffin embedding. Science 71: 103-104.
- . 1934. Amines in cytological fixing fluids. Protoplasma 20: 473-482.

